Home Office

NON-TECHNICAL SUMMARY

Investigating tissue immunity in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Tissue immunity, Ageing, Autoimmunity, Neurodegeneration, kidney disease

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To improve our understanding of how immune cells work in different organs in response to infection and inflammation, and to determine how these tissue responses are influenced by age, sex and the microbiome (the bacteria, viruses and fungi that live in the gut, nose and lungs).

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The immune system mediates responses to infection and to tissue damage. Historically, the study of immune responses has focused on organs where immune cells develop or are activated. These are called 'lymphoid organs" and include bone marrow, thymus, spleen and lymph nodes. However, there is an increasing appreciation that some immune cells live in non-lymphoid organs, for example, the kidneys, gut and meninges (the membranes that line the brain), and these have been largely ignored in immunology research. These tissue immune cells are involved in local responses to injury and infections within organs, but our understanding of their function and interactions, how they change with age is unclear, and will be the focus of this work. We also don’t know how immune cells in the gut, or in other organs, are influenced by the microbiome, which can be radically altered by antibiotic treatment, or whether these tissue responses contribute to sex-differences in disease susceptibility is unclear,

This information is important to develop better treatments for diseases that are tissue focused, including in the gut (inflammatory bowel disease), kidneys, joints (rheumatoid arthritis) and brain (neurodegenerative diseases like Parkinson's Disease or dementia). These chronic diseases are currently incurable, affect patients for decades, and have a significant impact on their lives and ability to work, with substantial health-economic implications. For example, dementia affects 1 million people in the UK, and is an ever-increasing problem for an ageing population, affecting 1 in 11 people over 65 years of age. We know that infection and inflammation in peripheral organs such as the gut and kidney can accelerate the progression of the neurodegenerative diseases that cause dementia but we don’t know why or how that happens. Our work will shed light on the mechanisms by investigating how immune challenges in one organ affect immune responses in a distant organ, potentially identifying new treatment strategies. Some of these diseases also have a strong bias to one sex. For example, systemic lupus erythematosus (SLE for short) is a disease that leads to inflammation in the kidneys, and is more common in women, with a 9:1 female to male ratio. In contrast, kidney disease caused by high blood pressure is more common in men. Understanding the mechanisms that drive these sex-based differences will help develop more personalised therapies.

What outputs do you think you will see at the end of this project?

We anticipate that this project licence will generate important **new knowledge** about tissue immune responses and how they are affected by age and the microbiome, and how they differ between sexes.

We will ensure that the information generated is widely disseminated, and have a strong track record in producing **publications** containing our work, including in the highest impact journals. For example, our previous project licence we produced data that was included or informed more than 40 manuscripts. We would anticipate a similar publication output.

In addition, we will also produce **'methods' papers or book chapters** to ensure our experience of best practice is available to the field.

Our lab also has expertise in using new technologies that generate information about how the genetic code of cells is translated into action, by measuring genetic messenger molecules called 'RNA'. This information is called 'transcriptomics' and includes tens of thousands of RNA measurements. We will make these data available for future use by researchers in **public repositories**, as well as in **open access browsers** that ensure investigators without computational or analysis skills can use the information, as we have done previously.

We may also file for **patents** where we identify potential new treatments.

Who or what will benefit from these outputs, and how?

This project will improve our understanding of tissue immune responses in different parts of the body, of relevance to a number of diseases where the benefits from our research outputs will be realised by **researchers**, **clinicians** and **patients**. This includes diseases in:

i. The intestine – our work will provide information that can be used to develop treatments for gut inflammation (conditions like inflammatory bowel disease), infection and cancers (long-term benefit).

ii. Kidney and bladder immunity – our studies will help identify strategies for the prevention and treatment for urinary tract infection, kidney injury (autoimmune and sterile) and chronic kidney disease. This will help prevent people from getting kidney failure, with important personal and health economic benefits; dialysis accounts for 2% of total NHS spending. Older people are more susceptible to urinary tract infections, we don't know why. Our studies will help find out if there are specific ways we can improve renal tract immunity in older people. Similarly, bladder infections are much more common in women than in men, affecting 50% of women at some point in their lives. We don't understand why, and work generated by this project will help to address this knowledge gap (short-term benefit).

iii. Musculoskeletal system - Joints are affected by autoimmune inflammation (eg, in rheumatoid arthritis) and by infections and they are also 'barometers' of systemic inflammation – with joint (and muscle) "ache" being common symptoms of systemic viral or bacterial infections. Our work will help delineate the cell type-specific mechanisms contributing to joint defence and inflammation, and sensing of circulating immune stimuli and will help us understand whether joint inflammation can influence immune activation in other organs (short-medium term benefit).

iv. Central nervous system organs – brain and meninges. There is currently limited information about the immune cells in the membranes lining the brain (the meninges). Our work will help determine how these cells defend the brain from infection, for example in diseases like meningitis and encephalitis

(short-term benefit). We will also assess how activation of immune cells in peripheral organs like the gut, kidney and lung, can affect meningeal and brain immune cells. We have already found surprising links between B and plasma cells in gut and CNS, but will extend this to include other immune cell types, delineating how this affects neuroinflammation and pathology in neurodegenerative diseases such as Parkinson's disease. This potentially opens the way for treatments that are delivered via the gut to modify CNS immunity, for example as oral vaccines that protect from meningitis (long term benefit).

How will you look to maximise the outputs of this work?

We will maximise our outputs via **research collaborations** and **dissemination of our work**.

Collaboration:

We have a strong track record for collaboration. In our previous project licence and work we participated in a number of collaborative projects with researchers locally, nationally and internationally.

We have on-going collaborations with some of these scientists, and will continue to operate in an open, collaborative manner, disseminating best practice and helping other in the field to maximise research outputs.

Dissemination

We disseminate information in open access papers as well as in methods and protocol papers, for example, we have previously made tissue dissociation methods available on Protocols.io.

We also use oral and poster presentations at conferences to disseminate our work to experts in the field. On average, our group members give at least one talk/lecture per month, including at international conferences.

In addition, we actively engage the public in our work via engagement with social media platforms and public lectures, ensuring a broad reach of our science.

Where we generate transcriptomic data, this will be made available for future use by researchers in public repositories, as well as in open access browsers.

Species and numbers of animals expected to be used

- Mice: Wild type and genetically modified mice: 18,900.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using mice for our research for several reasons:

Mice are genetically very similar to humans, with around 90% similarity in protein-coding genes, including many immune-related genes, They therefore provide a useful model for a variety of immune and infectious diseases, with many examples of this in the literature of how research in mice has identified important pathways in human disease.

Practically, there are many genetically modified mouse strains that enable the role of different immune cell types to be examined; mice can be genetically modified to remove a gene that is required for a specific cell type to develop, for example, removal of the Rag2 gene generates a mouse which has no B or T cells, two crucial immune cell types. More complicated genetic models are also possible, for example, a gene encoding a signalling molecule can be removed in only one cell type. There are also models that allow the removal or over-expression of genes to be switched on at a specific time or place, using harmless chemicals or light. We can also make mice that have been modified so that they have the same genetic variant that is found in some people with immune diseases. Finally, there are several mouse strains that have fluorescent immune cells, allowing their movement to be investigated in real time within a complex tissue environment.

As well as these different mouse strains, there is also a lot of prior experience and models of infection for the gastrointestinal and renal tract, that can be used in mice. This includes genetically modified pathogens that can be used to track specific immune cell responses.

Our project will use mice after birth, including aged mice, to investigate changes in immunity with age.

Altogether, these features of our model organism uniquely allow human-relevant immune responses across different tissues to be assessed and compared in a way that would be difficult in any other model organism.

Typically, what will be done to an animal used in your project?

We will use genetically modified animals, for example, mice that are either deficient in, or have too much of, an immune cell type, or and immune signal. Some of these mice will be aged to around 24 months to assess the effects on immune responses to tissue ageing. Most of these genetic modifications do not cause any symptoms in homeostasis (the healthy, unchallenged state).

In some cases we will change the immune system of the mouse by generating "bone marrow chimeras". This is done by giving irradiation to remove the bone marrow immune cells, followed by the introduction of genetically modified donor bone marrow. This allows mice to be generated that lack specific immune cells or immune signalling molecules without making a new mouse strain, avoiding lengthy breeding strategies. Following irradiation, mice may temporarily show reduced appetite and are at increased risk of infection. They will be weighed daily and food sweeteners/supplements offered to increase oral intake if required. They will also be assessed for evidence of infection and treated appropriately if needed.

We will also use genetically modified mouse models of neurodegenerative diseases (eg, Parkinson’s disease) to stimulate inflammation in the central nervous system (the brain and spinal cold). These mice develop disease as they age, and most show no symptoms until they are >14 months of age. At this time they may show reduced mobility and oral intake. In most cases, we will not age them to this

point, rather their tissues will be taken much earlier, when disease is evident if you examine the brain under a microscope, but mice do not have any overt symptoms.

We will use models of autoimmunity - these are diseases where the immune system attacks our own cells and tissues, rather than pathogens. There are inbred strains of mice that spontaneously develop autoimmune inflammation in the kidneys as they age, and we will use some of these models.

Mice will be challenged with immune stimuli (subcutaneous (under the skin), intranasal (via the nose), intravenous (into the vein), intra-peritoneal (into the abdominal cavity) or into the bladder via a catheter. These immune stimuli will include microbes, and effects on tissue immune cells will be assessed across different organs. Mice are regularly monitored post-challenge.

Mice will be exposed to gut inflammation (colitis) or infection (such as salmonella) by introducing bacteria or a substance (dextran sodium sulphate (DSS)) that causes inflammation orally in water or into the stomach. Following this, mice may develop weight loss (up to 15% of body weight). They may also develop pain and this will be treated with analgesia as needed.

Mice will be exposed to joint inflammation (arthritis) by introducing collagen and/or substance (adjuvant) that causes inflammation subcutaneously or intraarticularly. Following this, mice may develop weight loss (up to 20% of body weight). They may also develop pain and this will be treated with analgesia as needed.

For all protocols, we may monitor the immune response by taking urine or blood samples. Animals will experience mild and transient discomfort from blood sampling.

Experiments will be performed on young and old animals (including those of 12-24 months of age), and on both males and females.

Most of the challenge models are short (less than 1 week) but sometimes we will induce an immune response in one organ, wait for this to resolve and then re-challenge several weeks later, after recovery, with an infection in another organ.

What are the expected impacts and/or adverse effects for the animals during your project?

The expected impacts on animals vary according to the protocol.

For protocols involving challenge with pathogen or pathogen-associated molecule, mice may experience weight loss, piloerection (hair stands on end), hunched posture and reduced movement. There is no pain associated with these models. These models are short-lasting, typical duration 1-3 days.

For protocol involving intestinal inflammation or infection, mice experience weight loss, diarrhoea, and blood in their motions, as well as some abdominal pain. They may also become hunched and show reduced movement. The active phase of our models last around 7 days. After this, the infection is cleared (eg, salmonella) or the colitis resolves (DSS-induced colitis).

For protocols involving kidney injury/infection, mice may lose weight and may become hunched and show reduced movement. Kidney diseases are generally not associated with pain as the organ does

not contain pain neurons. Typical duration of these experiments is short (1-3 days). We do have a model of chronic pyelonephritis. In this case there is a repeat infection, but this is cleared spontaneously within 3-5 days.

For protocol involving arthritis, penetrance of collagen induced arthritis varies with strain and genetic background and is often difficult to predict. Mice may lose weight and may become hunched. However, experience gained using this model has shown that mice with inflamed paws move and forage well and we would not expect more than 20% of mice to reach moderate severity before the end of the experiment.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild 54%

Moderate 46%

Severe 0%

What will happen to animals used in this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We need to use animal models to understand how tissue immune cells in different organs respond to immune challenge and infection. Our focus is on gut, kidney, bladder, articular joints, brain and meninges. Each tissue has a different and complex mixture of structural cells and immune cells, and unique environmental cues that are generated by their homeostatic functions, eg, the kidney as some regions which have a high salt concentration. One part of the what we want to understand is how immune cells in these different organs influence each other, including by moving between one organ and another. All of this complexity means that these processes cannot be recapitulated using cells in a dish (in vitro), and an in vivo (live) model is needed.

Which non-animal alternatives did you consider for use in this project?

We have continued to develop a number of assays to replace and reduce animal use, including:

i. *In vitro* assays performed on murine cells immediately *ex vivo*, as well as on mouse and human cell lines.

ii. Use of human tissues. We have obtained ethical permission to use human kidneys donated for transplant that cannot be used and optimised protocols to extract tissue-resident immune cells. We have also set up a perfusion rig to interrogate the behaviour of human immune cells in the whole organ (kidney) *ex vivo* following different types of manipulation. This model will allow us to recapitulate some diseases in this ex vivo perfused human kidney and helps to replace and support mouse models of kidney injury.

We have also progressed a new ethics application to use kidney biopsies taken from patients with kidney diseases, which was approved in 2023. This helps to replace the use of disease models in mice.

We also obtain human intestinal and bladder tissue from our local Biorepository that retrieves tissue samples from organ donors.

For brain and meningeal tissues, we are working with an academic neurosurgeon and Neuropathologist to obtain human tissues from operations or post-mortem.

iii. Organoids – this system allows cells from an organ to be cultured in a dish, where they form a 3D structure that is similar to some aspects of how an organ works in real life. It is limited by the fact it usually only contains one of the cell types that are present in a real organ in vivo. We have developed a bladder organoid model and are collaborating with experts who have kidney, brain and intestinal organoid models.

Why were they not suitable?

The assays and tissue sources that we use for *in vitro* and *ex vivo* studies are useful, but they cannot completely re-create the complex tissue environment and many different cell types and cell interactions that occur *in vivo*. This is particularly important for our project, as in many cases we are trying to assess how an immune stimulus or infection in one organ in the periphery, for example, infection in the gut or kidney, affects immune cells in the central nervous system. These questions simply cannot be addressed without an *in vivo* animal model. Furthermore, when considering how tissue immune responses are influenced by ageing or the microbiome, *in vitro* cell stimulation or organoid assays are limited in what we can specifically assess. For example, ‘ageing’ a cell line or organoid in vitro cannot recapitulate the many complex signals tissue cells would receive *in vivo*.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimated number of animals are informed by the Home Office annual returns of procedure data from our current project licence. We will have similar numbers of researchers working on these projects and are likely to have a similar number of cre-flox complex crosses. We also have a similar number of protocols, and the majority are identical to our previous licence. Therefore, estimates based on our previous animal use should be reasonably accurate.

With regards to how the estimates of animal numbers were historically generated for specific experiments, we have support from the lab bioinformatician who has expertise in statistics and helped us with calculations using the observed endpoint variations in historical experiments in the lab, or from published studies, to calculate the minimum mouse number that can be used whilst ensuring that the results are statistically significant. For most protocols, our previous work has determined the difference in mean, the standard deviation of the experiments to achieve 5% significance level and therefore how many animals per observation are required to detect a significant difference.

We also use control animals/groups for all experiments (appropriately age and sex matched, ideally cohoused in order to minimise intragroup variability due to differences in microbiome. Where possible, we use blinding at all stages of our experimentation and analysis.

For situations where we are using a challenge or model that we have not used previously, and for which there is no published literature, we will use small pilot studies to ensure there is no excess harm to animals.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will minimise the number of animals used by carefully powering studies so that we use the minimum number of animals required to show a statistically significant effect. Power calculations and experimental design will be informed by the NC3Rs' experimental design guidance and experimental design assistant, utilising additional local statistical advice and support for randomisation and blinding where needed. Where randomised animal groups are needed, we will use appropriate methods, such as the Random Function in Excel.

We will also endeavour to minimise the introduction of systematic variations by ensuring that there are lab standard operating procedures (SOPs) for sample collection, and that there is in-house training for the specifics of tissue collection to ensure consistent fixation, quality of dissection, to minimise post-mortem delay and ensure proper storage conditions according to the experimental end-point assay.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

To optimise animal use, we will encourage tissue sharing. Within the group there is a tissue sharing scheme where all naïve wild type animals and most experimental animals are offered to other lab members, in case that organs that are not the main focus of the study can be utilised. Where possible we also collect additional tissues for storage (fixing/freezing) for future use, with documentation of stored tissues enabling other group members to utilise for method optimisation and training studies.

We also try and maximise the use of animals that are surplus to requirements for the original experiment by making them available to other researchers to address other biological questions. In particular stock, we have emailed the establishment 3R emailing list and shared these animals.

We have also provided cells from genetically modified animals for in vitro work to other researchers.

Our licence also includes the ability to perform non-terminal imaging to allow the same mouse to tracked repeatedly rather than requiring multiple mice at different time points.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The organ infection, inflammation and autoimmune models in our licence have been chosen because they are relevant to the human diseases we are investigating. In all cases, we use the protocol with the least severity to investigate the hypothesis under question. For immunisation and pathogen challenge models, we use the lowest dose possible that can generate the immune response under investigation. Similarly, we will use the shortest duration possible, determined by which part of the immune system is being investigated. This means that when assessing innate immune responses, very short experiments (sometimes even lasting only 4 hours) can be used. The challenge models will use a route of administration relevant to human vaccination, therapy or disease, for example, intravesical (into the bladder) delivery of bacteria using a urinary catheter to recapitulate human urinary tract infections, or skin injections, analogous to human vaccination. We also routinely provide environmental enrichment for animals and wherever possible house animals in groups to minimise distress.

To avoid breeding new knockout strains, we will use bone marrow chimeras. To create chimeric mice, irradiation is used to deplete the host cells before introducing donor cells which are then expanded within the host. This allows reconstitution of mice in such a way as to investigate a specific immune cell type and avoids the need to generate new mouse models or perform complex crosses which require many mice.

Why can’t you use animals that are less sentient?

Our project is focused on studying immunity in organs such as the kidney, gut, joints, bladder, meninges and brain. We will use post-natal mice, including aged mice. We cannot use mouse embryos as their immune system and organs are immature. This means that immune cells don't necessarily respond to immune challenges in the way that adult animals would. The structural cues present in organs are also different in embryos, for example, there is no high sodium environment in the kidney.

Non-mammalian animals are limited because their organs are very different structurally, for example, they may not even have synovial articular joints. Their immune cells are more rudimentary and have different receptors and responses to mammalian immune cells, meaning that they are not a good model for human organs or diseases.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We employ general measures to ensure mice are less stressed, including enriched environments, and housing mice in social groups. Where orally substances are given, we make sure they are as palatable as possible, using flavoured jelly, paste or milk shake liquid.

To ensure we minimise the severity and duration of protocols, we use frequent monitoring of relevant clinical parameters and endpoints, for example weight, piloerection, hunched posture, protein on urine dipstick. This ensures humane end-points are adhered to. We have score sheets that are specific for different protocols, for example, for gut inflammation and infection models, these sheets include documentation of weight, diarrhoea severity and whether there is blood in the stools. For kidney models, we use relevant biomarkers, for example blood urea or creatinine and urine protein, which can be monitored longitudinal, ensuring the shortest duration of experiments.

There are also specific protocols where we can minimise the distress to animals by combining procedures. For example, when the administration of more than one substance is required at the same time-point and using the same route, they will be combined in one procedure.

When generating bone marrow chimeras, mice may temporarily show reduced appetite and are at increased risk of infection following irradiation. In the intestinal infection/inflammation models, mice also may lose weight. In these protocols, mice are weighed daily, but we also modify their monitoring to increase the frequency if weight loss if more accelerated that initially appreciated. In addition, sweeteners are offered to increase oral intake.

We will also use genetically modified mouse models of neurodegenerative diseases (eg, Parkinson’s disease). These mice develop motor symptoms when they are old (>14 months of age), but in most of our experiments, we will not age them to this point, rather tissues will be taken much earlier, when disease is evident microscopically in the brain, but there are no overt symptoms that would distress the animals.

We are continually assessing how the procedures can be refined in order to minimise the discomfort that the animals may experience. Members of the lab holding personal licences who undertake procedures under the previous project licence meet regularly to review operational procedures. This has resulted in the implementation of changes that, for example, reduce the procedure time and hence the period of anaesthesia without compromising performance.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Will use the published guidelines to inform experiments, such as the ARRIVE and PREPARE guidelines: http://journals.sagepub.com/doi/full/10.1177/0023677217724823

As well as guidance from the Laboratory Animal Science Association, (LASA) https://www.lasa.co.uk/current_publications/

We will also follow the guidance available via NC3Rs publications and newsletters.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We are signed up to the local NC3R emailing list. We will also regularly check information on NC3Rs website. We receive regular newsletter and bulletins from our Named Information Officer and obtain the latest practical guidance from Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) to ensure we have up to date information about recommendations and advances in animal techniques.

We also regularly attends immunology meetings and conferences, including in neuroimmunology (eg, Keystone Neuroimmunology) , and these include presentations of new methodology and will adopt any new techniques that refine our experimental methods.